SEARCH FOR PROSTAGLANDIN-LIKE SUBSTANCES IN MARINE ORGANISMS

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The main facts regarding the present situation of research into prostaglandins (PG) are summarized in a monograph [1]. PG have now been found not only in higher land organisms, but also in lower marine organisms. An example of the latter is the discovery of PG in the gorgonian coral *Plexaura homomalla*, which is so far the only rich natural source of PG. As an adjunct to the many physiological, pharmacological, and clinical investigations of PG the search for accessible sources of these substances among marine organisms is particularly important.

The writers have analyzed 40 total prostaglandin extracts (PGX) of marine invertebrates belonging to six types for biological activity. This paper describes the results of these tests.

EXPERIMENTAL METHOD

Samples for isolation of total PGX were collected in Troitsa Bay of the Sea of Japan. The tissue was minced, weighed, and fixed in 95% ethyl alcohol with the addition of 0.5% acetic acid (40% aqueous). Tissue was separated from the extract by centrifugation. Extraction was carried out by the method described in [6]. Biological tests are among the most sensitive methods of PG determination [7].

Adult nulliparous rats weighing 150 g were used. The stage of the estrous cycle was determined by examination of vaginal smears. Uteri from rats only in the stage of diestrus or metestrus were used in the experiments. The rats were anesthetized with chloroform. From each uterine cornu a segment 1.5 cm was excised. The isolated organs were placed in 50-ml vessel filled with Tyrode solution at 37°C. To prevent any effect of acetylcholine, atropine was added ($1 \cdot 10^{-7}$ g/liter). Aliquots of the test total PGX were added to the solution surrounding the isolated uterine cornu and left in contact with the organ until maximal contraction was obtained. The muscular contractions were recorded on a chymograph.

EXPERIMENTAL RESULTS

The results in Table 1 demonstrate the widespread distribution of substances with a PG-like action among marine invertebrates: Of 40 species analyzed only three did not exhibit biological activity (these are indicated in the table by a minus sign). They belong to different types of invertebrates, and there is therefore no question of correlation with the position of the organism on the scale of evolution.

Most of the organisms tested had identical activity (indicated in Table 1 by a + sign), but five species of marine invertebrates exhibited higher activity (++). Total PGX of these animals induced muscular contractions in appreciably lower concentrations (20-40 times) than those from the remaining species.

Similar conclusions regarding the wide distribution of PG among marine invertebrates were drawn previously by Japanese workers [5] on the basis of a semiquantitative analysis of

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TABLE 1. Biological Activity of Total PGX

Phylum	Class	Species	Activity
Coelenterata	Scyphazoa	Cyanea arctica	+
	Anthozoa	Metridium senile fimbriatum	+
		Athopleura orientalis	+
Annelida	Polychaeta	Chaetopterus variopedatus	—
Arthropoda	Crustacea	Pagurus brachiomastus	+
		Pagurus middendorffi	+
		Cancer amphioctus	+
		Hapalogaster dentata	+
		Pachycheles stevensi	+
		Erimacrus isenbeckii	+
Mollusca	Gastropoda	Acmaea pallida	—
		Littorina squalida	+
		Littorina brevicula	+
		Littorina mandshurica	+
	Bivalvia	Modiolus difficilis	+
		Crenomytilus grayanus	+
		Anadara broughtoni	+
		Chlamys nipponensis	+
		Chlamys swifty	+
		Patinopecten yessoensis	+
		Mytilus edulis	+
		Callista brevisiphonata	+
		Mercenaria stimpsoni	++
		Spisula sachalinensis	+
		Peronidia venulosa	+
		Mya japonica	+
		Mactra sulcataria	+++
	Cephalopoda	Octopus dofleini	+
Echinodermata	Holothurioidea	Cucumaria japonica	+++
		Cucumaria fraudatrix	+
		Stichopus japonicus	+++
	Echinoidea	Strongylocentrotus nudus	+
		Strongylocentrotus intermedius	+
	Asteroidea	Patiria pectinifera	+
		Lysastrosoma anthosticta	—
		Distolasterias nipon	+
		Asterias amurensis	+
Tunicata	Ascidiacea	Aphelasterias japonica	+
		Halocynthia aurantium	+++
		Halocynthia roretzi	+

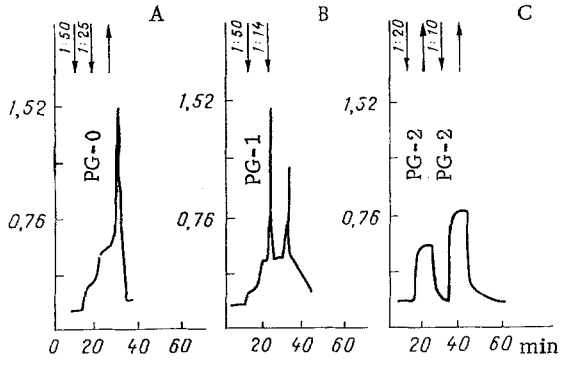

Fig. 1. Biological testing of PG fractions from the trepang *Stichopus japonicus* on the obturator muscle of *Cucumaria japonica*. A) Effect of total PG fraction; B) corresponding measurement for PG-1 (fraction from zone E); C) corresponding measurement for PG-2 (fraction from zone F). Numbers above arrow pointing downward indicate degree of dilution of original PG solution (25 mg/ml). Arrow pointing upward indicates washing with control solution (seawater). After washing with seawater, tension of muscle decreased to its initial value. Abscissa, time (in min); ordinate, force of contraction.

PG in 22 species of animals, of which 18 were marine invertebrates. In the present investigation the whole organism was used, whereas the Japanese workers studied individual organs (gonads, liver, gills, mantle, and so on). For biological testing they used the fundus of the rat stomach. The quantity of PG in the tissues of the animals they tested was very small, namely 1-45 ng/g wet weight of tissue.

Our results also confirmed data on the discovery of PG in eggs of the sea urchin *Strongylocentrotus intermedius* [2].

To verify the reliability of the data and to exclude any possible effect of other substances present in the PGX, those extracts which exhibited very high activity (+++) were subjected to preparative thin-layer chromatography (TLC). Biological tests of zones corresponding in chromatographic behavior to PGE_2 and $PGE_{2\alpha}$ confirmed the high activity of these extracts, and particularly high activity was exhibited by the trepang *Stichopus japonicus*.

Additional confirmation of the biological activity of total PGX from the trepang was obtained on the obturator muscle of *Cucumaria japonica* [3, 4]. For this purpose the extract also was fractionated by preparative TLC and zones corresponding in their chromatographic behavior to PG were tested. The results obtained on the obturator muscle of *Cucumaria japonica* are shown in Fig. 1.

The screening of this large number of marine invertebrates thus demonstrates the virtually universal presence of PG-like substances. The results justify a more intensive study of these substances from particular organisms.

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HEPATOCYTE PROLIFERATION AFTER BCG STIMULATION OF THE MONONUCLEAR PHAGOCYTE SYSTEM

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Stimulation of the Kupffer cells by the polysaccharide prodigiosan sharply enhances hepatocyte proliferation in rats after partial resection of the liver [1]. It was considered interesting to study how the hepatocytes regenerate during activation of the mononuclear phagocyte system (MPS) and, in particular, the Kupffer cells, by other methods. It must be emphasized that *Mycobacterium tuberculosis* effectively stimulates MPS and also directly stimulates the Kupffer cells [5, 9]. Disinhibition of the functions of the MPS reaches its peak when macrophages initially activate BCG, and later, to potentiate their secretion, old tuberculin is injected [2, 10]. The aim of the present investigation was to study the early stages of regeneration of the liver after repeated injections of preparations of *M. tuberculosis*.

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